

ADMET & DMPK 2(3) (2014) 179-184; doi: [10.5599/admet.2.3.47](https://doi.org/10.5599/admet.2.3.47)

Open Access : ISSN : 1848-7718

<http://www.pub.iapchem.org/ojs/index.php/admet/index>

Original scientific paper

## Electrochemical Synthesis, Structure Elucidation and Antibacterial Evaluation of 9a-aza-9a-chloro-9a-homoerythromycin A

Zoran Mandić<sup>a,\*</sup>, Gorjana Lazarevski<sup>b</sup>, Zlatko Weitner<sup>c</sup>, Predrag Novak<sup>d</sup>, Nataša Maršić<sup>e</sup>, Ana Budimir<sup>f</sup><sup>a</sup> University of Zagreb, Faculty of Chemical Engineering and Technology, Marulićev trg 19, HR-10000, Zagreb, Croatia<sup>b</sup> Ilica 134a, HR-10000, Zagreb, Croatia<sup>c</sup> Hrvatskih iseljenika 1, HR-10000, Zagreb, Croatia<sup>d</sup> University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102a, HR-10000, Zagreb, Croatia<sup>e</sup> Hraste and Partners Attorneys at Law, Ribnjak 40, HR-10000, Zagreb, Croatia<sup>f</sup> University of Zagreb, School of Medicine, Salata 3, 10 000 Zagreb\*Corresponding Author: E-mail: [zmandic@fkit.hr](mailto:zmandic@fkit.hr); Tel.: +385-1-4597-164; Fax: +385-1-3733-570

Received: July 20, 2014; Revised: August 26, 2014; Published: September 16, 2014

### Abstract

Electrochemical synthesis, structure elucidation and antibacterial evaluation of 9a-aza-9a-chloro-9a-homoerythromycin A were carried out. It was found that the anodic oxidation of 9a-aza-9a-homoerythromycin A via electrogenerated reactive chlorine species leads to the chlorination of lactam nitrogen in high yield provided the pH of the reaction mixture is maintained above 3. NOESY spectra reveal the existence of the mixture of two conformational families in the solution, the "folding-out" conformer being slightly more abundant comparing to 9a-Aza-9a-homoerythromycin A. The chlorine substitution of lactam hydrogen resulted in improved antimicrobial potency against *Streptococcus pyogenes* PSCB0542, *Moraxella catarrhalis* ATCC 25238, *Haemophilus influenzae* ATCC 49247 and *Enterococcus faecalis* ATCC 29212.

### Keywords

azithromycin; electrochemical chlorination; N-chloro lactam; antibacterial activity

### Introduction

9a-aza-9a-homoerythromycin A, **1**, belongs to a class of 15-membered ring macrolides which are important intermediates for the preparation of novel antibacterial compounds with improved antibiotic properties. It consists of 15-membered aglycone ring with cladinose and desosamine sugars attached to it at positions 3 and 5, respectively. The preparation of **1** is described in the literature [1].

As a part of the electrochemical derivatisation of macrolide antibiotics [2-6], we carried out the oxidation of **1** with electrogenerated reactive chlorine species in order to introduce chlorine atom into the lactam nitrogen. N-chlorolactam derivative should exhibit conformational changes in comparison with the starting compound what might in a greater or lesser extent affect biological activity. In addition, N-

chlorolactam derivative is a source of amydil radical [7] making it an important precursor for novel compounds with biological activity.

Although *N*-chloroamides and *N*-chlorolactams can be prepared by the number of chemical methods, the literature on the electrochemical halogenation of amides or lactams is sparse. Krishnamoorthy et al. [8] conducted a thorough evaluation of optimal conditions for the electrochemical bromination and chlorination of succinimide. They found that the reaction is highly sensitive on experimental parameters. Lyalin and Petrosyan [9] succeeded in preparative electrosynthesis of sodium salts of *N*-chloroamides of arylsulfonic acids.

In this paper we report on the electrochemical halogenation of **1**, thorough structural characterization and biological evaluation of the resulting product.

## Experimental

### Electrochemical synthesis of **2**

Electrochemical oxidation was carried out under constant current condition in the conventional two-compartment electrochemical cell. Compound **1** (20-100 mg) was dissolved in 20 ml of 0.1 mol dm<sup>-3</sup> LiCl solution in methanol and solution was electrolyzed with the constant current of 2.5 mA cm<sup>-2</sup>. Working electrode (anode) was platinum sheet and as cathode served graphite rod. The electrolysis was carried out at room temperature and the anolyte was stirred with a magnetic stirrer. The progress of the electrolysis was monitored by thin layer chromatography. After the completion of the electrolysis, the anolyte is separated from the reaction cell, evaporated to a dry residue and subjected to chromatography on a silica gel column (CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH-NH<sub>4</sub>OH = 90 : 9 : 1.5). The title compound is obtained in 60% yield with the following spectral data:

MS (ES<sup>+</sup>): *m/z* 797.4 (calc. 797.43);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  = 3444, 2973, 2937, 1731, 1650, 1463, 1381, 1169, 1055, 1011, 736; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  = 2.67 (1H, H-2), 3.54 (1H, H-3), 1.92 (1H, H-4), 3.72 (1H, H-5), 3.33 (1H, t, H-8), 4.81 (1H, m, H-10), 3.58 (1H, d, H-11), 1.38 (3H, d, H-10-CH<sub>3</sub>), 4.85 (1H H-13); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  = 44.1 (C-2), 71.8 (C-3), 41.7 (C-4), 78.8 (C-5), 30.8 (C-8), 179.1 (C-9), 52.0 (C-10), 11.2 (C-10-CH<sub>3</sub>), 71.8 (C-11).

### NMR

All 1D and 2D (<sup>1</sup>H, APT, gCOSY, gHSQC and gHMBC) NMR spectra were recorded at ambient temperature on the Avance DRX500 spectrometer working at 500.13 MHz for <sup>1</sup>H and equipped with a 5 mm diameter inverse detection probe with z-gradient. The sample concentration in CDCl<sub>3</sub> was 20 mg ml<sup>-1</sup> with TMS as the internal standard.

1D <sup>1</sup>H and APT NMR spectra were obtained with 4000 Hz and 32000 Hz spectral window, respectively, using 64K data points. Digital resolution was 0.12 Hz and 0.95 Hz per point, respectively.

2D gCOSY spectra were acquired with a sweep width of 6666 Hz in both dimensions into 2K data points with 512 increments. Spectra were zero-filled in the f1 dimension to 1K and processed using an unshifted sine bell window function. Digital resolution was 3.26 Hz per point and 16.58 Hz per point in f2 and f1 dimensions, respectively.

The inverse <sup>1</sup>H-<sup>13</sup>C correlation experiment gHMQC was recorded at 125.77 MHz using data matrices of 2K x 256 with 4 scans and processed with a shifted sine bell window function and linear prediction. HMBC spectra were recorded using a transfer delay for the evolution of long range C-H couplings of 60 ms with 256 increments into a matrix of 4Kx2K data points, with a sweep width of 7000Hz in f2 dimension and

31500 Hz in f1 dimension. Digital resolution was 1.7 Hz per point and 30.70 Hz per point in f2 and f1, respectively.

### Antibiotic Susceptibility Test

Antibiotic susceptibility data given in Table 1 were obtained by microdilution test in Mueller-Hinton media as described by NCCLS [10] except that test substances and standards were dissolved in DMF (Merck). Also for *Streptococcus* medium, blood was substituted with 5 % horse serum.

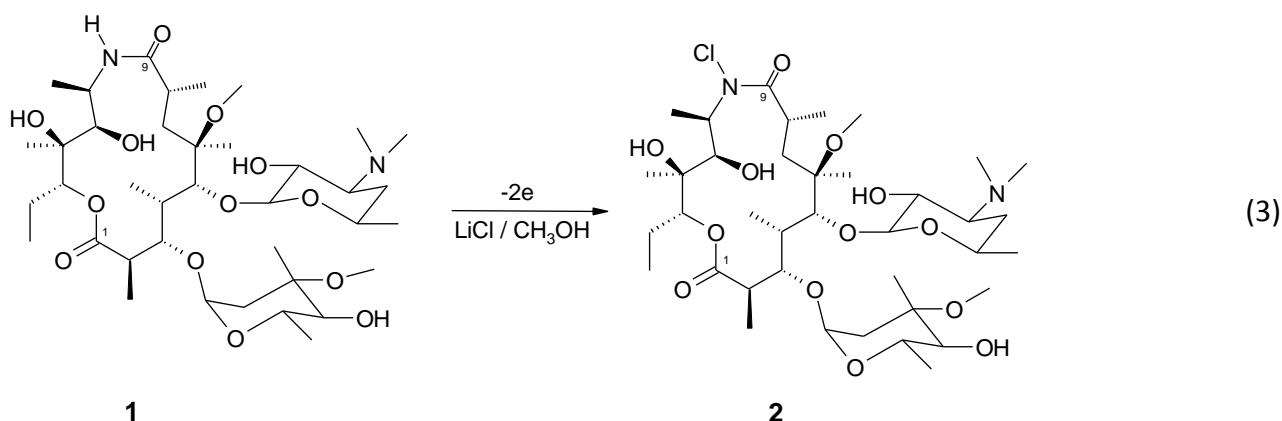
## Results and Discussion

### Formation of **2** by indirect electrochemical oxidation

Oxidation of chloride ions in methanolic solutions gives rise to the formation of reactive chlorine species as a powerful chlorinating agent. In the first step of the reaction dihalogen is formed which rapidly disproportionates into methyl hypochlorite,  $\text{CH}_3\text{OCl}$ , and hydrochloric acid (equations 1 and 2). The formation of N-Cl bond is an electrophilic substitution reaction and is believed to proceed via direct transfer of Cl from methyl hypochlorite to lactam nitrogen (equation 3) as in the case of chlorination of  $\alpha$ -amino acids by  $\text{NaOCl}$  in aqueous media [11].



The crucial feature of the electrochemical chlorination of **1** is that the formation of **2** is sensitive to pH of the solution and it was necessary to maintain the pH during electrolysis above 3. The anolyte acidifies during the electrolysis which is due to *in-situ* formation of hydrochloric acid and, in addition, to the less than 100% current efficiency of dihalogen formation. Namely, a small fraction of current is spent on oxygen evolution resulting in the liberation of protons. When the pH drops below 3, the equilibrium of reaction 2 is displaced to the left and the reaction stops. Therefore, in order to carry out the chlorination reaction in high yield, the pH of the reaction mixture was continuously adjusted by adding a few drops of the NaOH solution.



### Structural elucidation

The chemical structure of the compound **2** was determined by means of IR and NMR spectroscopies and MS spectrometry. IR spectrum has shown no band characteristic for N-H  $\delta$  stretching vibration at  $1530\text{ cm}^{-1}$  (amide II) observed for the starting compound **1**. A broad band in the region  $800\text{--}600\text{ cm}^{-1}$  assigned to N-H wagging disappeared as well. These findings indicate that there is no hydrogen atom attached to the lactam nitrogen and that the reaction took place at 9a position. In the MS spectrum a precursor ion peak was observed at  $m/z$  797.4 which is exactly by 34 Da higher than the mass of the starting compound. Isotopic pattern of precursor ion and some fragment ions revealed that chlorine atom is present in the molecule.

The carbon and proton chemical shifts of **2** were assigned by the combined use of the standard one- and two-dimensional NMR experiments. A disappearance of the lactam proton at 6.24 ppm as observed for **1** provides further evidence for chlorine substitution at nitrogen. Accordingly, changes in proton and carbon chemical shifts of the neighbouring atoms took place (Table 1). A characteristic down-field effect of atoms H8, H10, H11 and H10Me was observed due to the presence of the chlorine atom. Changes in neighbouring carbon atoms chemical shifts (both up- and down-field shifts) are the consequence of the chlorine substitution. In the APT spectrum the carbon multiplicity pattern is the same as that observed for the parent compound which excludes the substitution at the carbon atom.

Conformational studies on macrolides [12-14] have shown the existence of two major conformational families: folded-out and folded-in, referring to the outward and inward folding of the macrocycle ring fragment C3-C5. Those studies demonstrated that vicinal coupling constants  $^3J_{\text{H2H3}}$  and NOE proton-proton contacts H4-H11 and H3-H11, respectively, can serve as good indicators of the ring folding. In that respect, lower values of  $^3J_{\text{H2H3}}$  (2-3 Hz) and H3-H11 NOE cross peaks indicate the folded-in conformations whereas much higher coupling constant values (up to 10 Hz) and H4-H11 NOE contacts are characteristic for the folded-out ones. The ratio of the two conformational families was found to be dependent on the solvent and temperature. Recent results have shown that the ribosome-bound conformations of some 6-O-methyl homoerythromycins were found to be very similar to the free ones with some small differences observed [15].

X-ray structural analysis showed that **1** adopted a folded-in conformation in the solid state as was also the case in solution [15]. There was no indication of intramolecular hydrogen bonding involving the lactam nitrogen atom. According to the  $^3J_{\text{H2H3}}$  value measured for the solution of **2** (4.5 Hz) the abundance of the folded-out conformers has slightly increased in comparison with **1** (5.5 Hz) as a consequence of a chlorine substitution. Both H3-H11 and H4-H11 NOE cross-peaks were observed in the NOESY spectrum of **2** which pointed towards a mixture of the two conformational families.

**Table 1.** Chemical shift change from the starting compound, **1**, to product **2**

| atom  | chemical shift change,<br>ppm |
|-------|-------------------------------|
| H8    | 2.23 $\rightarrow$ 3.33       |
| H10   | 4.16 $\rightarrow$ 4.81       |
| H11   | 3.22 $\rightarrow$ 3.58       |
| H10Me | 1.16 $\rightarrow$ 1.38       |
| C8    | 35.4 $\rightarrow$ 30.8       |
| C9    | 177.1 $\rightarrow$ 179.1     |
| C10   | 45.0 $\rightarrow$ 52.0       |
| C10Me | 13.5 $\rightarrow$ 11.2       |

### Antibiotic susceptibility evaluation

The comparative MICs of **1** and **2** against different gram-positive and gram-negative pathogens are presented in Table 2. It appears that **2** possesses slightly improved potency against some respiratory pathogens. **2** was eight times more potent than **1** against *S. pyogenes* PSCB0542, and twice as active against *M. catarrhalis* ATCC 25238, *H. influenzae* ATCC 49247 and *E. faecalis* ATCC 29212. However, against erythromycin-susceptible *Streptococcus pneumoniae* compound **2** slightly lost its potency comparing to **1**. Against the rest of tested strains (*Staphylococcus aureus* PSCB0329, *S. aureus* PSCB0538, *S. aureus* PSCB0330, *S. aureus* PSCB0331, *S. pneumoniae* PSCB0328, *S. pneumoniae* PSCB0326, *S. pyogenes* PSCB0543, *S. pyogenes* PSCB0544, *S. pyogenes* PSCB0545, *E. coli* ATCC 25922) **2** had equal potency as **1**.

According to the NCCLS breakpoints for azithromycin in *S. pyogenes*, the change in MIC from 1 to 0.125 is significant, and therefore the new compound, unlike **1**, could be adequate for treatment of infections caused by these strains. In *H. influenzae*, breakpoint according to NCCLS is 4 µg/ml, and for clarithromycin is 8 µg/ml, and MIC 16 is interpreted as intermediary resistant.

Resistance to macrolides in gram-positive organisms whether inducible or constitutive, is due to methylation of an adenine residue in rRNA in 50S ribosomal subunit. 23S RNA is binding site for macrolides. The equal potency of **1** and **2** against macrolide-resistant gram-positive strains (strains with MLS and M phenotype), whether it was constitutive or inducible was expected, since the slight conformational difference between **1** and **2**, probably had no effect on the mechanism of action.

**Table 2.** MIC values (µg/ml) for **1** and **2**

| Strains                          | Phenotype | <b>1</b> | <b>2</b> |
|----------------------------------|-----------|----------|----------|
| <i>S. aureus</i> PSCB0329        | eryS      | 4        | 4        |
| <i>S. aureus</i> PSCB0538        | iMLS      | >64      | >64      |
| <i>S. aureus</i> PSCB0330        | cMLS      | >64      | >64      |
| <i>S. aureus</i> PSCB0331        | M         | >64      | >64      |
| <i>S. pneumoniae</i> PSCB0541    | eryS      | ≤0.125   | 0.25     |
| <i>S. pneumoniae</i> PSCB0328    | cMLS      | >64      | >64      |
| <i>S. pneumoniae</i> PSCB0326    | M         | 64       | 64       |
| <i>S. pyogenes</i> PSCB0542      | eryS      | 1        | 0.125    |
| <i>S. pyogenes</i> PSCB0543      | iMLS      | 64       | 64       |
| <i>S. pyogenes</i> PSCB0544      | cMLS      | >64      | >64      |
| <i>S. pyogenes</i> PSCB0545      | M         | 64       | 64       |
| <i>M. catarrhalis</i> ATCC 25238 |           | 0,25     | ≤0.125   |
| <i>H. influenzae</i> ATCC 49247  |           | 16       | 4        |
| <i>E. faecalis</i> ATCC 29212    |           | 16       | 4        |
| <i>E. coli</i> ATCC 25922        |           | >64      | >64      |

### Conclusions

Electrochemical indirect oxidation of 9a-Aza-9a-homoerythromycin A leads to the chlorination of lactam nitrogen, a novel compound in the class of macrolide antibiotic. The conformation of the chlorinated product in solution indicates more abundant presence of the “folded-out” conformer comparing to starting compound. In addition, it possesses slightly improved potency against some respiratory pathogens. However, the compounds described in this paper are not considered to be suitable candidates for drug development or to become lead compounds.

## References

- [1] G. Lazarevski, G. Kobrehel, Ž. Kelnerić, US Patent, 6,110,965, (2000)
- [2] Z. Mandić and N. Lopotar, *Electrochem. Comm.*, **7** (2005) 45-48
- [3] D. Iveković, N. Lopotar, K. Brajša, Z. Mandić, *Eur. J. Pharm. Sci.*, **18** (2003) 323-328
- [4] Z. Mandić, Z. Weitner, M. Ilijaš, *J. Pharm. Biomed. Anal.*, **33** (2003) 647-654
- [5] Z. Mandić, A. Narađa, P. Novak, K. Brajša, M. Đerek, D. Iveković, *J. Antibiot.*, **55** (2002) 807-813
- [6] Z. Mandić, A. Narađa, N. Lopotar, Lj. Duić, D. Iveković, M. Tkalčec, *J. Antibiot.*, **52** (1999) 1143-1145
- [7] B. Daoust and J. Lessard, *Tetrahedron*, **55** (1999) 3495-3514
- [8] S. Krisnamoorthy, R. Kanakam Srinivasan and M. Noel, *Bull. Electrochem.*, **16** (2000) 544-550
- [9] B.V. Lyalin and V.A. Petrosyan, *Russ. J. Electrochem.* **31** (1995) 1146-1150
- [10] Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. M100-S19. Vol 29. No 3. Clinical and Laboratory Standards Institute, 2009.
- [11] X.L. Armesto, M. Canle L., J.A. Santaballa, "α-amino acids chlorination in aqueous media", *Tetrahedron*, **49** (1993) 275-284.
- [12] J. R. Everett, J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, (1987) 1659-1667
- [13] P. Novak, Z. Banić Tomišić, P. Tepeš, G. Lazarevski, J. Plavec, G. Turkalj, *Org. Biomol. Chem.* **3** (2005) 39-47.
- [14] P. Novak, I. Tatić, P. Tepeš, S. Koštrun, J. Barber, *J. Phys. Chem. A* **110** (2006) 572-579.
- [15] N. Košutić-Hulita, D. Matak-Vinković, M. Vinković, P. Novak, G. Kobrehel, G. Lazarevski, *Croat. Chem. Acta*, **74** (2001) 327-341.

©2014 by the authors; licensee IAPC, Zagreb, Croatia. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>)

